(12) UK Patent Application (19) GB (11) 2 283 239 (13) A

(43) Date of A Publication 03.05.1995

(21) Application No 9322353.5

(22) Date of Filing 29.10.1993

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(51) INT CL[6]
C07K 14/705 , C12N 15/12 // C07K 16/28 , C12Q 1/02 1/68 , (C12N 15/12 C12R 1:91)

(52) UK CL (Edition N)
C3H HB7P H644 H649 H655 H656 H686
C6Y Y406

(56) Documents Cited
Eur.J.Biochem; Vol 224, 1994, pages 489-495
Biochemical and Biophysical Research Communications, Vol 201 No. 2, 1994, pages 894-901
J.Allergy Clin.Immunol.,Vol.93, No.1, part 2, 1993 page 215, Abstract 314 Biochemical and Biophysical Research Communications,Vol.197, No.3, 1993, pages 1601-1608 Biochemical and Biophysical Research Communications, Vol.190 No.1, 1993, pages 294-301
Biochemical and Biophysical Research Communications,Vol.178, No.3, 1991, pages 1386-1392 Proc.Natl.Acad.Sci,USA, Vol. 88, 1991, pages 11515-11519

(58) Field of Search
UK CL (Edition M) C3H HA5 HB7M HB7P
INT CL[5] C07K 15/06 , C12N 15/12
ONLINE: WPI, BIOTECH (DIALOG)

(54) Human H1 histamine receptor

(57) There is disclosed the isolation of the human H1 histamine receptor protein, the gene which encodes this protein and nucleic acid probes therefor. Vectors are detailed which are adapted for the expression of this receptor on the surface of CHO cells. There are disclosed methods for determining ligand binding, detecting the presence of human H1 histamine receptor on the surface of a cell, drug screening and detecting the presence of mRNA coding for the protein in a cell.

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FIG. 1

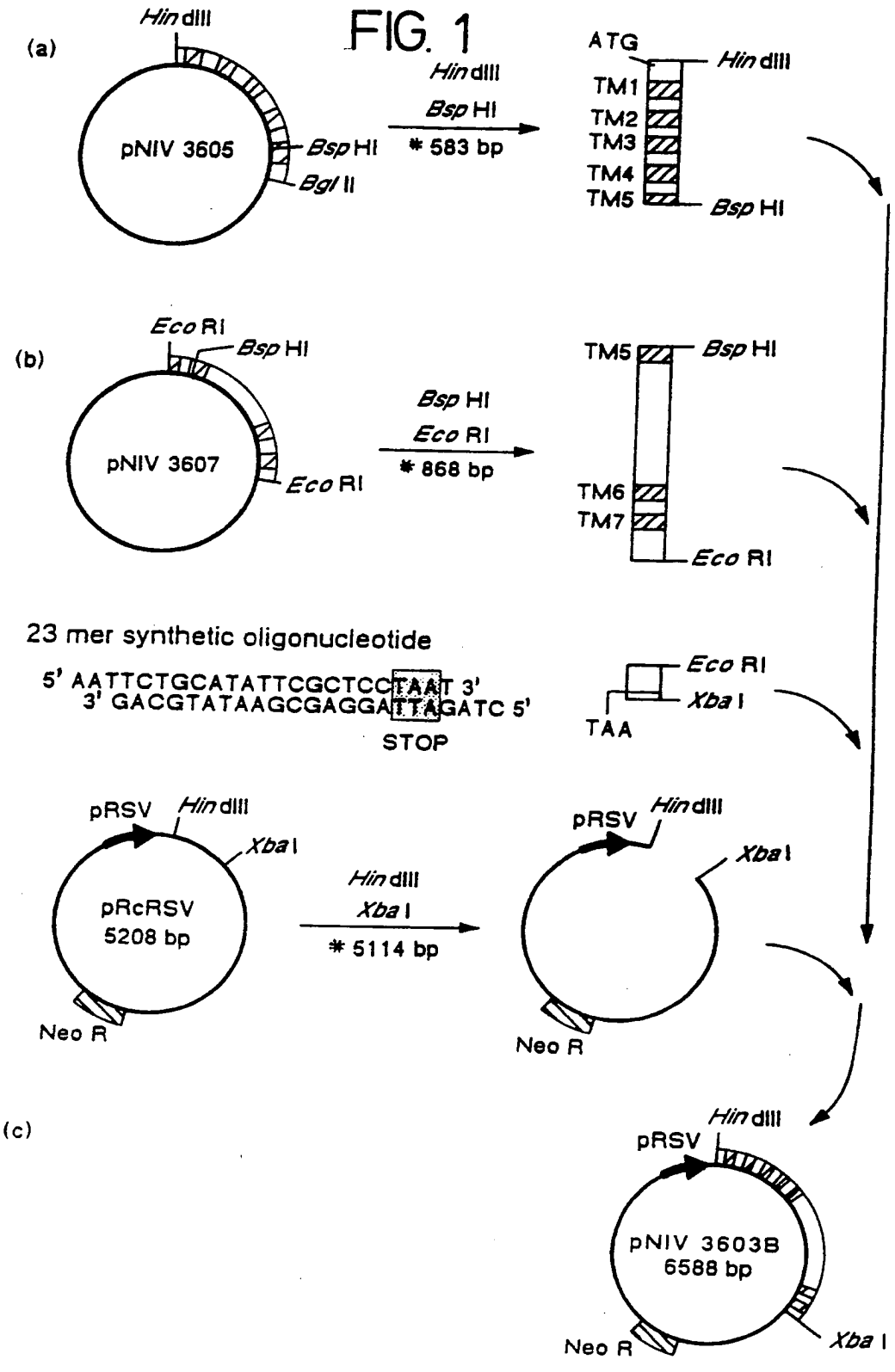
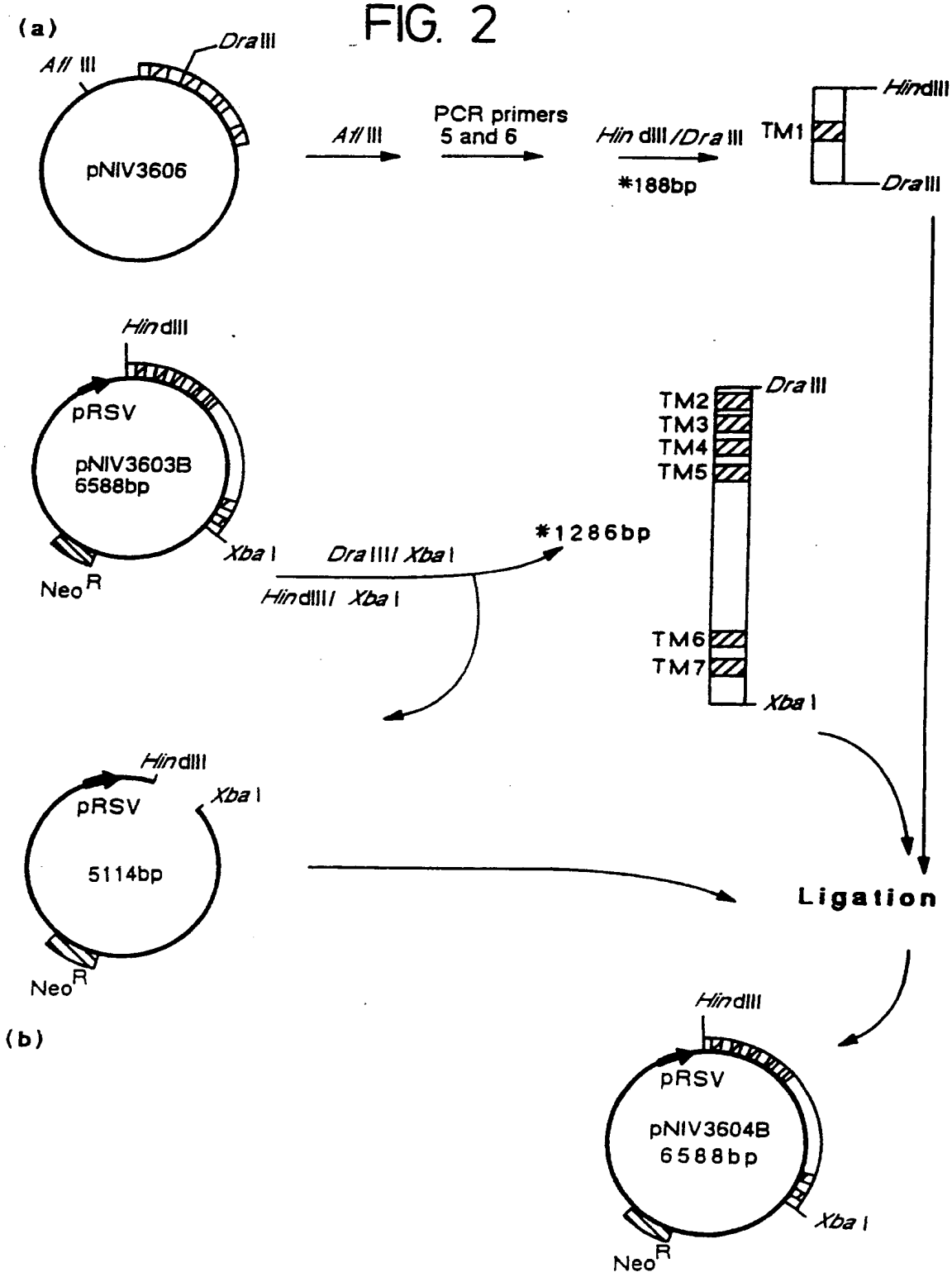

(a)
Hin dIII
pNIV 3605
Bsp HI
Bgl II
Hin dIII
Bsp HI
* 583 bp
ATG
Hin dIII
TM1
TM2
TM3
TM4
TM5
Bsp HI
(b)
Eco RI
Bsp HI
pNIV 3607
Eco RI
Bsp HI
Eco RI
* 868 bp
TM5
Bsp HI
TM6
TM7
Eco RI
23 mer synthetic oligonucleotide
5' AATTCTGCATATTCGCTCCTAAT 3'
3' GACGTATAAGCGAGGATTAGATC 5'
STOP
Eco RI
Xba I
TAA
pRSV
Hin dIII
Xba I
pRcRSV
5208 bp
Neo R
Hin dIII
Xba I
* 5114 bp
pRSV
Hin dIII
Xba I
Neo R
(c)
Hin dIII
pRSV
pNIV 3603B
6588 bp
Neo R
Xba I

FIG. 2


(a)
Dra III
Afl III
pNIV3606
Afl III
PCR primers
5 and 6
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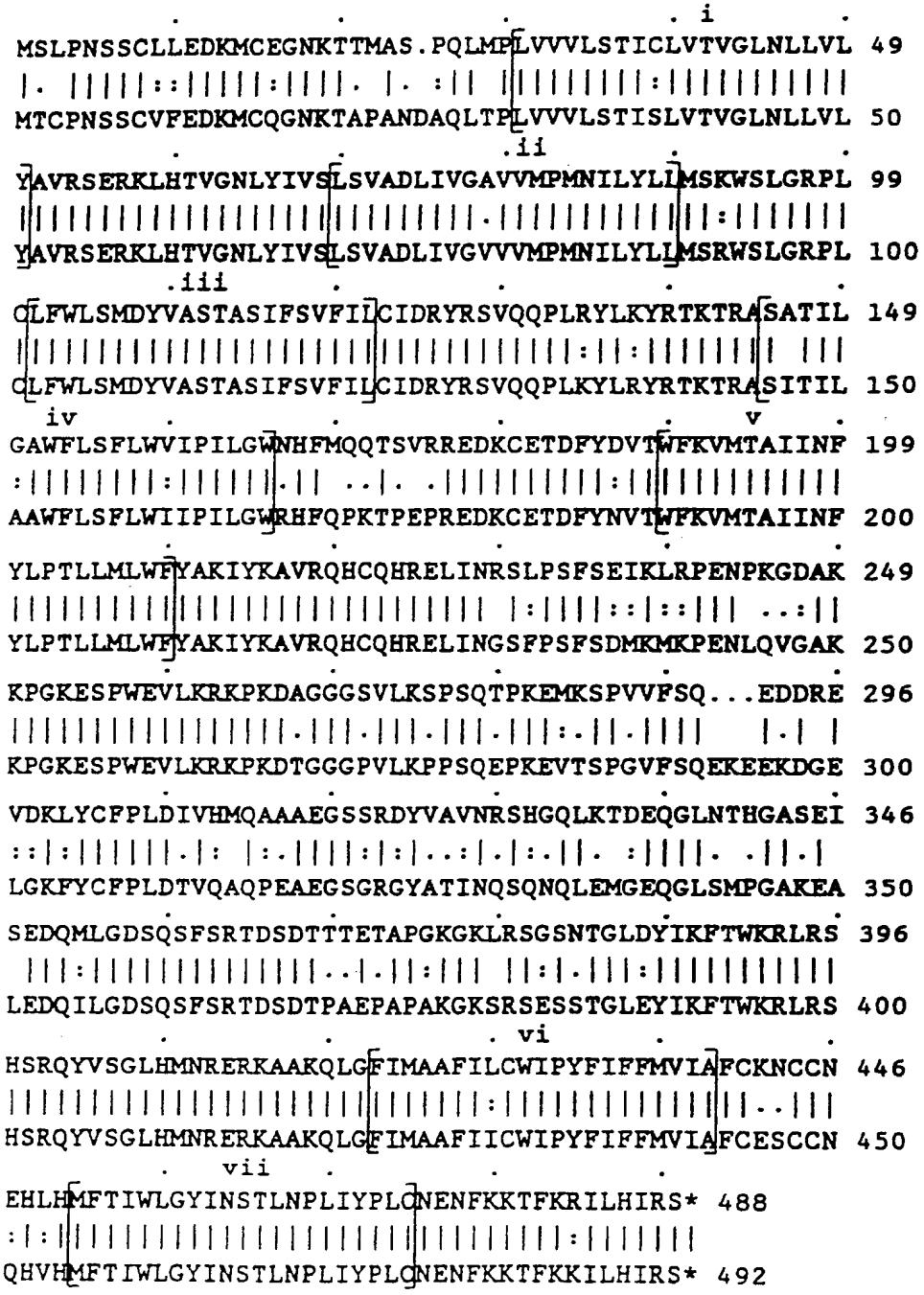
*188bp
TM1
Hind III
Dra III
Hind III
pRSV
pNIV3603B
6588bp
Xba I
Neo R
Dra III/ Xba I
Hind III/ Xba I
*1286bp
TM2
TM3
TM4
TM5
Dra III
TM6
TM7
Xba I
Hind III
pRSV
5114bp
Xba I
Neo R
Ligation
(b)
Hind III
pRSV
pNIV3604B
6588bp
Xba I
Neo R

FIG. 3

Comparison of the primary structure of human (upper line) and bovine (lower line) histamine H_1 receptors.



DNA encoding a human histamine H_1 receptor

DESCRIPTION

Pharmacological studies, and more recently gene cloning, have established that multiple receptor types exist for histamine (M.E. PARSONS, Scand. J. Gastroenterol. suppl. 180,(1991), 46 - 52; E. E. HAAKSMA et al., Pharmacol. Ther. 47(1), (1990), 73 - 1041).

Three types have been described so far, i.e. the H_1, H_2 and H_3 receptors. Receptor antagonists have been used in the therapy of many allergic diseases, including urticaria, allergic rhinitis, pollenosis and bronchial asthma. In addition, histamine receptors are involved in the mediation of smooth muscle contraction, contraction of terminal venules, catecholamine release from adrenal medulla and mediation of neurotransmission in the central nervous system. The existence of multiple receptor types provides one mechanism by which histamine can elicit distinct cellular responses. The variation in cellular response can be achieved by the association of individual receptor types with different G proteins and different signaling systems.

The individual receptor types reveal characteristic differences in their abilities to bind a number of ligands but the structural basis for the distinct ligand-binding properties is not known. Physiological and pharmacological studies have been carried out to try to characterize particular biological functions, or anatomical locations, for these histamine receptor types, but this was not very successful. In addition, the biochemical mechanisms by which these receptors transduce signals across the cell surface have been difficult to ascertain without having well-defined cell populations which express exclusively one histamine receptor type.

Like many other G protein-coupled receptors, histamine receptors have a seven-transmembrane configuration. While all the histamine receptors are recognized by histamine, they are pharmacologically distinct and are encoded by separate genes. These receptors are coupled to different second messenger pathways via guanine nucleotide regulatory proteins (G proteins). Among the histamine receptors, the H_1 receptor transduces the signal through calcium ion mobilization via an increase in the intracellular inositol 1,4,5-triphosphate level and the H_2 receptor activates adenylate cyclase. Nothing is known so far about the

intracellular signaling system used by the H_3 receptor.

Radioligand filtration binding techniques have been used to characterize the histamine receptor family. Using these methods, the three major classes of histamine receptors have been described, H_1, H_2 and H_3. These differ in their selectivity for drugs (J.R. RAYMOND et al., J. Biol. Chem. 266(1), (1991), 372-379; I.GANZ et al., J. Biol Chem. 267, (1992), 20840-20843; M. YAMASHITA et al., Biochem. Biophys. Res. Commun. 177, (1991), 1233-1239; J. C. SCHWARTS, Annales de l'Institut Pasteur/actualités, 2(1991), 101-104). H_1 receptors can be labeled selectively with [^{3}H]mepyramine and [^{125}I]iodobolpyramine, H_2 receptors can be labeled selectively with [^{3}H]tiotidine and [^{125}I]iodoaminopotentidine, and H_3 receptors with [^{3}H]-(R)-α-methylhistamine.

Within the H_1, H_2 and H_3 receptor family there may be several subtypes, but these have not yet been identified.

Applicant has cloned a human histamine H_1 receptor cDNA, which has been transfected into an heterologous expression system, producing a membrane protein with binding properties consistent with its characterization as a histamine H_1 receptor.

A variety of structural features which are invariant in the family of histamine receptor proteins were present in the new histamine receptor protein molecule. The greatest homology was found between the cloned human histamine H_1 receptor and the bovine histamine H_1 receptor (M. YAMASHITA et al., Proc. Natl. Acad. Sci. USA, 88, (1991), 11515-11519).An overall identity of approximately 82 % was observed, while the identity within the transmembrane regions alone was approximately 96 %.

The cloned receptor shares sequence and structural properties with the family of receptors spanning the lipid bilayer seven times. These receptors namely include the α- and ß-adrenergic receptors (H.G. DOLHMAN et al., Biochemistry 26, (1987), 2657) and the muscarinic cholinergic receptors (T.I. BONNER et al., Science 237, (1987), 527). All of them appear to transduce extracellular signals by interaction with guanine nucleotide-binding proteins (G proteins) (H.G. DOLHMAN et al., Biochemistry 27, (1988), 1813).

The present invention provides an isolated nucleic acid molecule encoding a human histamine H_1 receptor and also an isolated protein which

is a human histamine H_1 receptor.

The invention also provides vectors such as plasmids comprising DNA molecules encoding a human histamine H_1 receptor, for example a plasmid designated pNIV3604B.

Additionally, the present invention provides vectors adapted for stable expression in bacterial, yeast, insect or mammalian cells which comprise DNA molecules encoding a human histamine H_1 receptor and the regulatory elements necessary for expression of the DNA molecules in the cell.

The present invention further provides stably transfected Chinese hamster ovary (CHO) cell lines, for example a CHO cell line designated CHO_{3604B}.

In addition, the invention provides DNA probes useful for detecting nucleic acid encoding a human histamine H_1 receptor, comprising a nucleic acid molecule of at least about 15 nucleotides having a sequence complementary to a coding sequence included within the DNA sequence shown in SEQ ID No:4.

This invention also provides a method for determining whether a ligand which is not known to be capable of binding to a human histamine H_1 receptor can bind to such a histamine H_1 receptor.

The invention also concerns antibodies, polyclonal and monospecific, directed to a human histamine H_1 receptor, and particularly, monoclonal antibodies directed to epitopes of a human histamine H_1 receptor present on the surface of a cell and having an amino acid sequence included within the amino acid sequence shown in SEQ ID No:4.

The invention concerns a method to detect the presence of a human histamine H_1 receptor on the surface of a cell.

The invention also concerns a method of screening drugs to identify drugs which specifically interact with, bind to and activate the human histamine H_1 receptor.

The invention, finally, discloses a method for detecting human histamine H_1 receptor subtypes by using the cDNA described in SEQ ID No:4 as a probe on mRNA present in various tissues and organs.

Figure 1 shows the construction of the expression plasmid pNIV3603B encoding a hybrid bovine/human histamine H_1 receptor.

Figure 2 shows the construction of the expression plasmid pNIV3604B encoding the human histamine H_1 receptor.

Figure 3 gives the comparison of the primary structure of human (upper line) and bovine (lower line) histamine H_1 receptors. Amino acid sequences (one-letter code) are aligned to optimize homology. Between the two structures, a vertical line means no difference, a double point means a polarity-conservative substitution, one point means a polarity-semiconservative substitution and a blank indicates a complete difference between amino acids. The putative transmembrane domains are indicated in brackets , and identified by Roman numerals above the upper line. Numbers refer to amino acids positions.

The one-letter abbreviations for amino acid residues are :
A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophane; and Y, tyrosine.

The present invention provides an isolated nucleic acid molecule encoding a human histamine H_1 receptor. The DNA molecule is preferably a complementary DNA molecule. The invention also provides a DNA or a cDNA molecule having a coding sequence substantially the same as the coding sequence shown in SEQ ID No:4.

The invention provides an isolated protein which is a human histamine H_1 receptor. Such a receptor protein has substantially the same amino acid sequence as the amino acid sequence shown in SEQ ID No:4.

The invention provides a means to obtain human histamine H_1 receptors by expressing DNA encoding the receptor in a suitable host, such as bacteria, yeast, insect or mammalian cells, using methods well known in the art, and recovering the histamine H_1 receptors after being expressed in such a host, again using methods well known in the art.

The invention provides vectors comprising DNA encoding a human histamine H_1 receptor or DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID No:4. Vectors may be plasmids, cosmids or bacteriophages. Preferably, plasmids will be used according to the invention. An example of a plasmid carrying cDNA having a coding sequence substantially the same as that shown in SEQ ID No:4 is the plasmid designated pNIV3604B, which is described in greater detail hereinafter.

The invention further provides plasmids adapted for expression in bacterial, yeast, insect or mammalian cells which comprise a) DNA encoding a human histamine H_1 receptor or DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID No:4, and b) the regulatory elements necessary to express such DNA in the host cells cited above. Those skilled in the art will readily appreciate that numerous plasmids may be constructed utilizing existing plasmids and adapted, as appropriate, to carry the regulatory elements necessary to express the DNA in mammalian cells. In particular, it may be of interest to include on the expression plasmid a genetic amplification module such as the dihydrofolate reductase (DHFR) expression cassette, described by CONNORS et al., (DNA 7, (1988) 651-660). The presence of the DHFR expression cassette on the expression plasmid offers the possibility to expose transfected cells to increasing concentrations of methotrexate thereby selecting effectively those cells which carry multiple copies of the integrated expression plasmid and thus express higher levels of the

desired protein. Numerous mammalian cells may be used including, for example, the mouse fibroblast cell NIH3T3, HeLa cells and CHO cells. An example of a plasmid carrying such a genetic amplification module and adapted for the expression of a DNA molecule having a coding sequence substantially the same as the coding sequence shown in SEQ ID No:4 is also the plasmid pNIV3604B, which is described more fully hereinafter.

The invention provides expression plasmids used to transfect mammalian cells, for example CHO cells, comprising plasmids adapted for expression in these cells which comprise DNA encoding a human histamine H_1 receptor or comprise DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID No:4. In one preferred embodiment, the present invention provides CHO cells transfected with the plasmid designated pNIV3604B. This cell line is designated CHO_{3604B}.

The present invention further provides a method to determine whether a ligand, such as a known or putative drug, which is not known to be capable of binding to the human histamine H_1 receptor, can bind to the human histamine H_1 receptor. This method comprises a) contacting a mammalian cell with the ligand, under conditions permitting binding of ligands known to bind to this receptor, b) detecting the presence of any of the ligand bound to the human histamine H_1 receptor and thereby determining whether the ligand is capable to bind to a human histamine H_1 receptor. An example of a mammalian cell is a CHO cell comprising a plasmid carrying a cDNA molecule encoding a human histamine H_1 receptor whose amino acid sequence is substantially the same as that shown in SEQ ID No:4.

The invention still further provides a method of detecting the presence of mRNA coding for a human histamine H_1 receptor in various cells, tissues and organs. The method consists of obtaining total mRNA from cells, tissues and organs, using well known methods, contacting the mRNA so obtained with the cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID No:4 under hybridizing conditions, detecting the presence of mRNA hybridized to the cDNA and thereby detecting the presence of mRNA coding for a human histamine H_1 receptor in cells, tissues and organs.

The present invention also provides DNA probes useful for detecting in a sample nucleic acid encoding a human histamine H_1 receptor. Such probes comprise nucleic acid molecules of at least 15 nucleotides having

a sequence complementary to sequences included within the DNA sequence shown in SEQ ID No:4. Those skilled in the art know the technology of nucleic acid probes and will appreciate that such probes may vary in length and may be labeled with a detectable label, for example, radioisotopes or chemiluminescent dyes, to facilitate the detection of the probe.

The invention provides antibodies directed against a human histamine H_1 receptor. These antibodies may be serum-derived or monoclonal and can be prepared according to well-known methods. For example, CHO cells expressing the human histamine H_1 receptor may be used as immunogens to raise such antibodies. Alternatively, synthetic peptides, constructed on the basis of the amino acid sequence shown in SEQ ID No:4, may be prepared using commercially available machines.

Still further, the invention provides a method of detecting the presence of human histamine H_1 receptors on the surface of a cell. The method comprises a) contacting the cell with a monoclonal or serum-based antibody directed to an exposed epitope on the histamine H_1 receptor under conditions permitting binding of the antibody to the receptor, and b) detecting the presence of the antibody bound to the cell and thereby the presence of a human histamine H_1 receptor on the surface of the cell. Such a method is useful in determing whether a given cell is defective with respect to the expression of histamine H_1 receptors on the cell surface.

Finally, the invention provides a method of screening drugs to identify drugs which specifically interact with, bind to and activate the human histamine H_1 receptor on the surface of a cell. A plurality of drugs, known or putative, can be tested by contact with a mammalian cell line expressing the human histamine H_1 receptor. An example of a mammalian cell line is the CHO cell line designated above as CHO_{3604B} which is suitable for such experiments.

Specifically, this invention thus relates to the first isolation of a human cDNA clone encoding a human histamine H_1 receptor by using the amplification technique known as Polymerase Chain Reaction (R. K. SAIKI et al., Science 239, (1988),487-491) and also to the expression of a histamine H_1 binding site in CHO cells by transfecting the cells with the cDNA from plasmid pNIV3604B for example. A mammalian cell line, CHO_{3604B}, expressing a human histamine H_1 receptor at the cell surface has been constructed, as determined by pharmacological methods, thus

establishing the first well-defined cultured cell line with which to study the human histamine H_1 receptor and the response of cells to the activation of the receptor by known or putative ligands.

Response systems are obtained by coupling the human histamine H_1 receptor encoded by the isolated cDNA molecule to an appropriate second messenger generating system which includes, but is not limited to, phosphoinositide hydrolysis, adenylate cyclase or ion channels. The response system is obtained by transfection of the cDNA of the invention into a suitable host cell containing the desired second messenger system. Such a host system is isolated from pre-existing cell lines or is generated by inserting appropriate components of second messenger systems into cells expressing the human histamine H_1 receptor.

The system described above provides means to test the ability of ligands to activate the receptor encoded by the cDNA molecule of the invention. Transfection systems, such as those described above, are useful as living cell cultures for competitive binding assays between known and candidate drugs and ligands, which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor expressed by transfected cells are also useful for competitive binding assays in allowing the measurement of binding affinity and efficacy. Such a transfection system constitutes a "drug discovery system", useful for the identification of natural or synthetic compound with potential for drug development that can be further modified or used directly as therapeutic compound able to activate or inhibit the natural functions of the human histamine H_1 receptor of the invention. The invention thus identifies an individual receptor protein and tests whether pharmacological compounds interact with it for use in therapeutical treatments.

In summary, the invention identifies for the first time a human histamine H_1 receptor protein, its amino acid sequence and its corresponding cDNA. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents and new therapeutic or diagnostic assays for this new receptor protein, its associated mRNA or its associated genomic DNA.

The invention will be better understood by reference to the examples which follows and which are only illustrative of the invention.

EXAMPLE 1

Isolation, cloning and sequencing of the human histamine H_1 receptor cDNA.

On the basis of the nucleotide sequence of the bovine histamine H_1 receptor (M. YAMASHITA et al., Proc. Natl. Acad. Sci. USA, 88, (1991), 11515-11519), oligonucleotide primers were synthesized and used to amplify, by the polymerase chain reaction technique (R.K. SAIKI et al., Science 239, (1988), 487-491), the corresponding human histamine H_1 receptor cDNA, starting from a human lung total cDNA library (Clontech, U.S.A., Quick clone). The sequences of the different primers used are represented in SEQ ID No: 5 to SEQ ID No: 10.

Using primer 1 (SEQ ID No: 5), which corresponds to the 5' end of the coding sequence of the bovine histamine H_1 receptor DNA, and primer 2 (SEQ ID No: 6), which corresponds to the complementary DNA sequence which falls within the 5th transmembrane region of the bovine histamine H_1 receptor, a DNA sequence of 661 bp was amplified. It encompasses, between the two primers, sequences corresponding to a fragment of the human histamine H_1 receptor cDNA. The two primers contain 4 bases upstream from the Hind III site and 8 bases dowstream from the Bgl II site. These 12 bases improve the hybridization of the primers and facilitate the digestion with these restriction enzymes to give a DNA sequence of 649 bp represented in SEQ ID No: 1. This fragment was subcloned in the cloning vector pSP73 (Promega, U.S.A.) and is designated pNIV3605 (Figure 1(a)).

A 643 bp DNA fragment, recovered from pNIV3605 by digestion with Hind III and Bgl II, was then used to probe a λgt11 human lung cDNA library (Clontech, U.S.A.), according to techniques well known in the art. A total of 152,000 clones were screened and one positive clone was isolated and characterized by restriction endonuclease mapping and DNA sequence analysis. This clone, λgt11 (16H51b), was shown to carry sequences encoding a large fragment of the human histamine H_1 receptor cDNA. The cDNA insert in this clone spans about 1300 bp, starting 115 bp upstream from the sequence corresponding to the 5^{th} transmembrane region of the human H_1 receptor and ending with about 280 bp of non-coding sequences downstream to a TAA stop codon. The DNA sequence of cDNA insert in pNIV3605 and the cDNA insert in λgt11(16H51b) are overlapping. Together they reconstitute the complete coding sequence for the human histamine H_1 receptor cDNA with the exception of the 39 first bases at the 5' end which, by construction, were of bovine origin.

For construction convenience, a 992 bp Eco RI fragment was recovered from clone λgt11(16H51b) and subcloned in the cloning plasmid pUC18 (Pharmacia), yielding plasmid pNIV3607. This plasmid carries the cDNA sequence coding for the 5th transmembrane region up to the end of the human histamine H_1 receptor, but lacks the last 22 bp including the TAA stop codon (SEQ ID No:2 and Figure 1(b)).

In order to isolate and identify the missing 5' end of the human histamine H_1 receptor cDNA, total human lung cDNA (Quick clone, Clontech, U.S.A.) was amplified using primers 3 (SEQ ID No: 7) and 4 (SEQ ID No: 8) which correspond respectively to the 5' leader non-coding sequence of bovine histamine H_1 receptor cDNA and to the complementary sequence of the 4th transmembrane region of the human histamine H_1 receptor. The resulting amplified DNA fragment was isolated, subcloned into the cloning vector pUC18 (Pharmacia), yielding plasmid pNIV3606 (Figure 2(a)) By DNA sequence analysis, this plasmid pNIV3606 was shown to carry 18 bp of a non-coding sequence at the 5' end followed by the coding sequence for the 5' end of the human histamine H_1 receptor starting with the ATG initiation codon followed by 462 bp up to the fourth transmembrane region (SEQ ID No:3).

The DNA sequence information obtained from the cDNA inserts of pNIV3605, λgt11(16H51b), pNIV3607 and pNIV3606 allowed the reconstruction of the complete cDNA sequence coding for the human histamine H_1 receptor. This sequence and the corresponding deduced amino acid sequence of the protein are shown in SEQ ID No:4.

An open reading frame extending from an ATG initiation codon at position 1 to a stop codon at position 1464 can encode a protein of 487 amino acids in length. A comparison of this protein sequence with previously characterized receptors indicates that it is a new member of a family of molecules which span the lipid bilayer seven times and couple to guanine nucleotide regulatory proteins (the G protein-coupled receptor family). A variety of structural features which are invariant in this family were present in the new histamine receptor protein molecule. The greatest homology was found between the new human histamine H_1 receptor protein molecule and the bovine histamine H_1 receptor (M. YAMASHITA et al., Proc. Natl. Acad. Sci. USA 88, (1991), 11515-11519). An overall identity of approximately 82 % was observed, while the identity within the transmembrane regions alone was approximately 96% (Figure 3). A difference in length between the bovine and the human H_1 receptors can be

observed: the bovine receptor protein contains 491 amino acids whereas the human receptor protein has only 487 amino acids. The differences are apparent in the N-terminal part and in the third intracellular loop regions which are usually the less conserved among receptors of the G protein-coupled receptor family. Transmembrane regions are indicated between brackets; they were predicted according to the method of EISENBERG et al. (J. Mol. Biol., 179, (1984), 125-142). These regions are 21 amino acid residues in length.

All experimental protocols used above have been fully detailed in the books "Current Protocols in Molecular Biology" (AUSUBEL et al., Green Publishing Associates and Wiley Intersciences, New York, 1992) and "Molecular Cloning" (SAMBROOK et al., Cold Spring Harbor Laboratory Press, U.S.A., 1989) and in the protocols of the product manufacturers (Clontech, U.S.A.).

Nucleotide sequence analysis was done by the Sanger dideoxynucleotide chain-termination method (S.SANGER et al., Proc. Natl. Acad. Sci. USA, 74, (1977) 5463-5467), on denatured double-stranded DNA templates using Taquence (US Biochemical Corp., Cleveland, Ohio, USA).

EXAMPLE 2

Construction of a hybrid bovine/human histamine H_1 receptor.

Starting from plasmid pNIV3605 (prepared in example 1), which carries the 643 bp cDNA fragment (Figure 1 (a)), a 583 bp DNA fragment flanked by Hind III and Bsp HI restriction sites was isolated. This fragment encodes the initiation codon (Met 1), 12 amino acids of the bovine histamine H_1 receptor and 179 amino acids of the human histamine H_1 receptor, ending in the 5th transmembrane region at amino acid residue 192. Note that the 5' leader sequence located between the Hind III site and the ATG initiation codon contains the stretch of nucleotides ACC which is the consensus sequence for initiation of translation (M. KOZAK, J. Biol. Chem. 266, (1991), 19867-19870).

Starting from plasmid pNIV3607 (also prepared in example 1), which carries the 992 bp cDNA fragment (Figure 1 (b)), a 868 bp Bsp HI-Eco RI DNA fragment was isolated corresponding to the sequence encoding the C-terminal part of the human histamine H_1 receptor, from amino acid residue 193 to amino acid residue 481 of the protein molecule.

A third DNA fragment was generated by the synthesis of two 23-mer complementary oligonucleotides, which by annealing provide flanking Eco RI and Xba I restriction sites. The synthetic DNA fragment encodes the last six amino acid residues 482 to 487 of the receptor molecule and provides a TAA stop codon upstream from the Xba I restriction site. The three fragments described above were ligated together with the eukaryotic expression vector pRcRSV (British Biotechnology Ltd., United Kingdom) previously cut with Hind III and Xba I restriction enzymes, yielding the final recombinant expression vector pNIV3603B which contains the Neo Selection Module (Neo ^{R}) expressing the neomycin resistance. This plasmid thus carries a DNA sequence encoding a hybrid bovine/human histamine H_1 receptor molecule having 487 amino acid residues (Figure 1(c)) and in which the 13 first amino acids are of bovine origin.

EXAMPLE 3

Construction and expression of the human histamine H_1 receptor in transfected mammalian cells

a) Vector construction

Plasmid pNIV3606 (see example 1), which carries the 483 bp cDNA fragment described in Figure 2(a), was linearized by digestion with Afl III and submitted for amplification to the polymerase chain reaction using primers 5 (SEQ ID No: 9) and 6 (complementary; SEQ ID No: 10). A 202 bp DNA fragment resulting from the amplification was obtained. After digestion with the enzymes Hind III and Dra III a 188 bp DNA fragment was obtained and purified. It is flanked by Hind III and Dra III restriction sites and carries a 5' leader non-coding sequence CCA upstream from the ATG initiation codon (Met 1) and the sequence encoding amino acids 2 to 60 of the human histamine H_1 receptor.

Starting from plasmid pNIV3603B constructed in example 2 (Figure 1), two fragments were isolated by digestion with either Dra III and Xba I or Hind III and Xba I.

The first fragment spans 1286 bp, is flanked by Dra III and Xba I restriction sites and codes for amino acid 61 to amino acid 487 of the human histamine H_1 receptor and includes a TAA stop codon.

The second fragment spans 5114 bp, is flanked by Hind III and Xba I restriction sites and corresponds to the pRcRSV plasmid, as described before in example 2. Ligation of the three fragments indicated above yielded the recombinant eukaryotic expression plasmid pNIV3604B which thus carries the DNA sequence encoding the complete human histamine H_1 receptor (487 amino acid residues, Figure 2(b)).

b) Production of stably transfected CHO cell lines

In order to confirm the functional identity of the newly isolated gene, plasmid pNIV3604B was transfected and expressed into CHO cells .

Plasmid pNIV3604B, linearized with Aat II, was transfected by electroporation (Gene Pulsor, Biorad, USA) into CHO K1 cells (ATCC accession No CCL61), using 20 µg DNA per 10^7 cells. (Alternatively, CHO DG44 dhfr$^-$ cells (G. URLAUB and L. A. CHASIN, Proc. Natl. Acad. Sci. USA 77, (1980), 4216-4220) are suitable for transfection). Cells were maintained in α MEM medium (Alpha Modified Eagle's minimal essential medium, GIBCO, USA) supplemented with ribonucleotides and desoxyribonucleotides, 5 % fetal calf serum and L-glutamine.

Conditions for transfection and growth of cells have been described

in detail in MOGUILEVSKY et al. (Eur. J. Biochem. 197, (1991) 605-614).

Selection of transfectants was done by supplementing the culture medium with neomycin (geneticin G418, 0.4 mg/ml : Gibco Laboratories, Grand Island, New York). Clones expressing geneticin resistance were selected.

c) Membrane preparation

Transfected geneticin-resistant CHO clones were subcultured in α MEM medium containing L-glutamine and supplemented with 5 % fetal calf serum. The cells were grown at 37°C in a humidified atmosphere of 5 % CO_2 and 95 % air.

Confluent cells were gently scraped with a rubber policeman and resuspended in phosphate buffered saline (PBS; 25 ml for 6 x 175 cm^2 flasks). All the subsequent operations were performed at 4°C. The cell suspension was centrifuged for 10 minutes at 500 g. The pellet was homogenized (10 strokes at 1000 rpm) in a 20 mM Tris-HCl (pH 7.4), 250 mM sucrose buffer (buffer A) using a Potter S homogenizer (Braun, Germany). The homogenate was centrifuged at 29000 g for 15 minutes. The resulting pellet was washed 2 more times under the same conditions. The crude membrane pellet obtained was resuspended and stored at -80°C in buffer A at a protein concentration of 6 to 8 mg/ml.

d) Binding Experiments on membranes prepared from the CHO_{3604B} clone.

Binding data were analysed by a non linear curve fitting technique using the appropriate equations to describe a one- or two-site model [G.A.WEILAND and P.B.MOLINOFF, Life Sci. 29, (1981), 313-330, P.B.MOLINOFF et al, Life Sci.29, (1981), 427-443; A.DE LEAN et al, Mol.Pharmacol. 21, (1982),5-16; J.R.UNNERSTALL in Methods in Neurotransmitter Receptor Analysis. Eds.H.I.Yamamura, Raven Press, New York, 1990, 37-68]. IC_{50} values were converted to K_i (equilibrium dissociation constant of the competitor) by applying the CHENG AND PRUSOFF equation [Y-C CHENG and W.H. PRUSOFF, (Biochem.Pharmacol. 22, (1973), 3099-3108].

1. [^{3}H]Mepyramine binding. Saturation studies.

Assays were performed with [^{3}H]mepyramine, a specific ligand (tracer) for histamine H_1 receptor type, according to R.S.L. CHANG et al., (J.Neurochem. 32, (1979), 1653-1663) and M.M.BILLAH et al., (J.Pharmacol.Exp.Ther. 252, (1990), 1090-1096). Briefly, membranes (300 µg proteins) were incubated in 500 µl (final volume) of 50 mM

Tris-HCl (pH 7.4) buffer containing 2 mM $MgCl_2$ and increasing concentrations from 0.2 to 20 nM of [^{3}H]mepyramine (21 Ci/ mmol, Amersham, Belgium). The assays were carried out at 37°C for 180 minutes. Receptor-bound [^{3}H]mepyramine was separated from the free ligand by rapid vacuum filtration of the samples over glass fiber filters (GF/C, Whatman, VEL, Belgium) presoaked in 0.05 % polyethylenimine in order to reduce the non specific binding of the tracer to the filter.

Adsorbed samples were washed four times with 2 ml of ice-cold 50 mM Tris-HCl (pH 7.4) buffer. The entire filtration procedure did not exceed 10 seconds/sample. Radioactivity trapped onto the filter was determined by liquid scintillation counting at 50-60 % efficiency. The non specific binding of [^{3}H]mepyramine was measured by the inclusion of 10 µM cetirizine or 2 µM triprolidine in the assay. Under these experimental conditions, the specific binding represented 73 ± 5 %.

[^{3}H]Mepyramine bound reversibly to the receptors expressed in the membranes of these CHO cells. Equilibrium was reached within 1 minute and the binding remained stable for at least 30 minutes. After an incubation of 180 minutes, approximately 40 % of the specific binding was lost. Complete dissociation of the tracer from its receptors was achieved within 5 minutes (kinetic constant k_{off} = 1.2 min^{-1}; $t_{1/2}$ = 0.6 min.).

Saturation curves for [^{3}H]mepyramine binding revealed a single population of binding sites displaying high affinity for the tracer. The dissociation constant of the tracer K_d and the maximum number of binding sites B_{max} are respectively 5.1 nM and 210 fmol/mg protein.

2. [^{3}H]Tiotidine binding.

[^{3}H]Tiotidine (87 Ci/mmol, New England Nuclear, Belgium) binding was performed essentially as described by Y.HATTORI et al., [Br.J.Pharmacol. 103, (1991), 1573-1579]. Briefly, membranes (300 µg protein) were incubated in 250 µl (final volume) of 50 mM Tris-HCl (pH 7.4) buffer containing 2 mM $MgCl_2$ and 6 nM of [^{3}H]tiotidine, a specific ligand (tracer) for histamine H_2 receptor type. The incubation was carried out at 25°C for 60 minutes. The filtration procedure is identical to the one described above for [^{3}H]mepyramine. Non specific binding was determined in the

presence of 100 µM ranitidine.

A K_d value of 10 nM was determined for [^{3}H]tiotidine binding to H_2 histamine receptors in guinea pig cerebral cortex, following the experimental conditions described above. So, at a concentration of radioligand of 6 nM, the tracer should label about 40 % of the total number of H_2 receptors eventually present in the CHO cells membranes, assuming a same K_d value for these receptors. No specific binding of [^{3}H]tiotidine could be detected on the membranes prepared from the CHO_{3604B} clone.

3. [^{3}H]N-alpha-methylhistamine

[^{3}H]N-alpha-methylhistamine (84 Ci/mmol, New England Nuclear, Belgium) binding assay was performed essentially as described by A.KORTE et al., Biochem.Biophys.Res.Commun. 168 (3), (1990), 979-986]. Briefly, membranes (300 µg proteins) were incubated in 500 µl (final volume) of 50 mM Tris-HCl (pH 7.4) buffer containing 2 mM $MgCl_2$ and 0.5 nM of [^{3}H]N-alpha-methylhistamine, a specific ligand (tracer) for histamine H_3 receptor type. The incubation was carried out at 25 °C for 60 minutes. The filtration procedure is identical to the one described above for [^{3}H]mepyramine. Non specific binding was determined in the presence of 10 µM thioperamide.

A K_d value of 0.6 nM was determined for [^{3}H]N-alpha-methylhistamine binding to H_3 histamine receptors in guinea pig cerebral cortex, following the experimental conditions described above. So, at the concentration used in the assay (0.5 nM), the tracer should label about 50 % of the H_3 receptors eventually present in the CHO cells membranes. No specific binding of [^{3}H]N-alpha-methylhistamine on the membranes prepared from the CHO_{3604B} clone could be detected.

4. [^{3}H]Mepyramine competition studies.

The H_1 type identity of the histamine receptors on the membranes prepared from the CHO_{3604B} clone was further asserted by competition experiments with various drugs including cyproheptadine, promethazine, triprolidine, hydroxyzine, (+)-chlorpheniramine, diphenhydramine and cetirizine which are known to be selective antagonists for histamine H_1 receptor type, ranitidine, which is a selective ligand for histamine H_2 receptor type and thioperamide which is a selective ligand for histamine H_3 receptor type.

Samples (300 µg protein) were incubated for 180 minutes at 37 °C with 4 nM of [^{3}H]mepyramine and increasing concentrations of drugs as described previously for [^{3}H]mepyramine binding assays. The data were analysed by non linear regression according to a one-site model. Histamine competition curves were further analysed according to a two-site model. The dissociation constant K_i and the Hill coefficient nH of the drugs tested are listed in Table I. The table shows the two average results obtained from two independant experiments done in duplicate. The competition curves with histamine were best fitted according to a two-site model. The values for histamine are the means of three experiments and the numbers given between brackets are the proportions of high and low affinity sites for histamine.

Table I

Inhibition of [^{3}H]mepyramine binding to a histamine H_1 receptor in CHO_{3604} transfected cells.

DRUGS	pK_i	nH
Cyproheptadine	10.3 - 10.1	0.95 - 1.22
Promethazine	9.6 - 9.5	0.87 - 1.08
Triprolidine	9.2 - 9.0	0.91 - 0.95
Hydroxyzine	8.6 - 8.7	0.97 - 1.02
(+)-Chlorpheniramine	8.5 - 8.6	0.93 - 1.00
Diphenhydramine	8.0 - 8.0	1.04 - 1.08
Cetirizine	7.9 - 8.2	0.96 - 1.12
Histamine	6.4 (60%) ¦ 5.1 (40%)	0.65
Thioperamide	4.0 - 4.0	0.97 - 0.93
Ranitidine	< 5.0 - < 4.0	not determined

These results show that cyproheptadine, promethazine, triprolidine, hydroxyzine, (+)-chlorpheniramine, diphenhydramine and cetirizine displayed high affinity towards the receptors labelled with

[^{3}H]mepyramine, whereas ranitidine, a H_2 selective drug was only a weak competitor. Hill coefficients close to 1.0 indicated that the drugs competed for an homogeneous class of receptors.

Thioperamide, a H_3 selective drug, competed only very poorly with [^{3}H]mepyramine, as demonstrated by its low pK_i value. The binding of histamine was complex as anticipated for an agonist interacting with a G protein-coupled receptor.

It is to be noted that histamine and all the H_1 antagonists tested completely displaced [^{3}H]mepyramine from all the receptor sites labelled by the ligand.

EXAMPLE 4

Tissular distribution of the human histamine H_1 receptor

Determination of the tissular distribution of the human histamine H_1 receptor is effected by hybridization experiments using the cDNA described in SEQ ID No:4, or part of it, as a probe and total mRNAs extracted from different tissues as targets. The experimental procedure, known as Northern blotting, is well known in the art and is fully described in "Current Protocols of Molecular Biology" (AUSUBEL et al., loc. cit.). In short, total mRNAs extracted from different tissues are separated by migration on an agarose gel, then transferred onto a nylon membrane. A commercially available membrane (Clontech, USA) carrying separated mRNAs from a variety of tissues and ready to use for hybridization was used as starting material. The cDNA probe, labeled with ^{32}P consists of a 1426 bp DNA fragment containing the coding sequence for amino acid 14 to amino acid 487 of the human histamine H_1 receptor. Hybridization of the probe to the membrane was performed at 42°C in the conditions recommended by the manufacturer. After hybridization at 42°C, two series of washings were performed determining increasing stringency conditions: first washing at 50°C and second washing at 55°C in the solution described in Table II. Then, the membrane was exposed for 5 days to X-ray films to permit visualization of the mRNA detected by the probe. Thanks to molecular weight standards incorporated into the membrane, it is possible to measure the size of the hybridizing mRNA.

Table II summarizes the results obtained in the experiments. It can be seen that mRNA molecules complementary to the probe are found in all tissues tested whether or not the hybridization conditions were stringent

or relaxed. However, salient features can be observed. Indeed, in the brain, a typical rather abundant 4.8 kb mRNA band was detected, which is absent in all other tissues. In addition, the distribution of hybridizing mRNAs varied from tissue to tissue, the abundance being maximal in the brain. There were also mRNAs of different size in the same tissue, this difference being most probably due to variations in the length of the 3' non-coding sequences and to the occurrence of different polyadenylation signals in the molecules. The type of experiment described above thus not only allows the identification of the human histamine H_1 receptor mRNA in various tissues but also offers a reliable and quick tool to identify putative tissular subtypes of the human histamine H_1 receptor mRNA.

Table II

Distribution and size of human H_1 receptor mRNAs in various tissues

		heart	brain	placenta	lung	liver	striated muscle	kidney	pancreas
(1)	Abundance	+	5+	4+	3+	±	2+	2+	±
	Size (in kb)		4.8						
		4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
				1.5					
(2)	Abundance	+	5+	4+	3+	±	2+	2+	±
	Size (in kb)		4.8						
		4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5

(1) : Hybridization at 42°C and washing in relaxed conditions : 2 x SSC; 0.05 % SDS; T=50°C.

(2) : Hybridization at 42°C and washing in stringent conditions : 0.1 x SSC; 0.1 % SDS; T=55°C.

5+ to ± : from "very abundant" to "low abundant"

2 x SSC : sodium citrate 0.03M and sodium chloride 0.3M, pH 7

0.1 x SSC : sodium citrate 0.0015M and sodium chloride 0.015M, pH 7

SSC : standart saline citrate

SDS : sodium dodecyl sulfate

EXAMPLE 5

Chromosomal location of the gene coding for the human histamine H_1 receptor.

Using two panels of somatic cell hybrids segregating either human or rat chromosomes, the gene encoding the human histamine H_1 receptor was assigned to human chromosome 3.

The procedure used has been extensively detailed before in the following publications: WATHELET et al, Somatic cell and Molecular Genetics 14, (1988), 415-426; SZPIRER et al, Genomics 10, (1991), 539-546 and SZPIRER et al, Genomics 11, (1991), 168-173.

Briefly, filter hybridization of DNA from human-rat somatic cell hybrids was performed using as a probe, a 1060 bp KpnI-XbaI fragment derived from plasmid pNIV3604B and labelled with ^{32}P by the random priming method. Comparison of the segregation of the histamine H_1 receptor gene with the human chromosome composition of each somatic cell hybrid revealed complete concordance for the presence or absence of a single human chromosome, i.e. chromosome 3.

EXAMPLE 6

Antibodies raised against the human histamine H_1 receptor

In order to generate antibodies directed to the human histamine H_1 receptor, a computer-based prediction of potential B epitopes was performed on the amino acid sequence shown in SEQ ID No:4, according to the algorithms of Kyte and DOOLITTLE and HOPP-WOODS, which are available in the computer menu GCG, program Peptide structure (The Genetic Computer Group, Madison, Wis. USA). On this basis, the following B epitope was identified:

5'- Met Gln Gln Thr Ser Val Arg Arg Glu Asp Lys Cys Glu Thr Asp
1 5 10 15

Phe Tyr Asp Val-3'.

This peptide sequence of 19 amino acids is located on the second extracellular loop of the human histamine H_1 receptor, at position 169 to 187 in the amino acid sequence shown in SEQ ID No:4. This peptide has been synthesized on an automatic peptide synthesizer (ABI model 430A), purified by HPLC, coupled to the tetanus anatoxin and injected to animals (rabbits and mice) to generate antibodies. Protocols are well known in the art and are fully described in "Current Protocols in Immunology" (J.E. COLIGAN et al., Green Publishing Associates and Wiley Intersciences, New York, (1991). Antibodies raised in animals against the B epitope described above are useful to detect the expression and localization of the human histamine H_1 receptor

protein, on the surface of the cell. Detection can be achieved by immunofluorescence assays, Western blotting or ELISA (see "Current Protocols in Immunology", J.E. COLIGAN et al., loc. cit.) and is independent of any biological activity (binding of ligands, activation) of the receptor protein.

Discussion

Applicant has cloned and characterized a cDNA molecule encoding a human histamine H_1 receptor. The expression of the cDNA clone in CHO cells results in the appearance of this type of receptor on the cell surface.

Binding competition studies on transfected CHO_{3604B} cell membranes with [^{3}H]mepyramine, a selective tracer for H_1 receptors and ligands recognized as H_1 selective drugs are consistent with histamine receptors of the H_1 type. The inability of ranitidine, a H_2 selective drug, or of thioperamide, a H_3 selective drug, to compete with [^{3}H]mepyramine, as well as the absence of binding with [^{3}H]tiotidine or with [^{3}H]N-α-methylhistamine, support the identification of the receptor expressed in the CHO_{3604B} clone as a histamine H_1 receptor.

SEQUENCE LISTING

NUMBER OF SEQUENCES: 10

(1) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 649 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: Lung

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Clontech, USA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 10..48
(D) OTHER INFORMATION: /partial
/product= "Bovine Histamine H1 Receptor"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..648
(D) OTHER INFORMATION: /partial
/product= "Human Histamine H1 Receptor"

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..48

(D) OTHER INFORMATION: /note= "Correspond to a part of the primer 1 (SEQ ID NO: 5:) used for amplification"

(ix) FEATURE:

(A) NAME/KEY: primer_bind

(B) LOCATION: 607..649

(D) OTHER INFORMATION: /note= "Correspond to a part of the primer 2 (SEQ ID NO: 6:) used for amplification"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ATG TGT GAG GGC AAC AAG ACC ACT ATG GCC AGC CCC CAG CTG ATG CCC        96
Met Cys Glu Gly Asn Lys Thr Thr Met Ala Ser Pro Gln Leu Met Pro
  1               5                  10                  15

CTG GTG GTG GTC CTG AGC ACT ATC TGC TTG GTC ACA GTA GGG CTC AAC       144
Leu Val Val Val Leu Ser Thr Ile Cys Leu Val Thr Val Gly Leu Asn
            20                  25                  30

CTG CTG GTG CTG TAT GCC GTA CGG AGT GAG CGG AAG CTC CAC ACT GTG       192
Leu Leu Val Leu Tyr Ala Val Arg Ser Glu Arg Lys Leu His Thr Val
        35                  40                  45

GGG AAC CTG TAC ATC GTC AGC CTC TCG GTG GCG GAC TTG ATC GTG GGT       240
Gly Asn Leu Tyr Ile Val Ser Leu Ser Val Ala Asp Leu Ile Val Gly
    50                  55                  60

GCC GTC GTC ATG CCT ATG AAC ATC CTC TAC CTG CTC ATG TCC AAG TGG       288
Ala Val Val Met Pro Met Asn Ile Leu Tyr Leu Leu Met Ser Lys Trp
65                  70                  75                  80
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TCA CTG GGC CGT CCT CTC TGC CTC TTT TGG CTT TCC ATG GAC TAT GTG      336
Ser Leu Gly Arg Pro Leu Cys Leu Phe Trp Leu Ser Met Asp Tyr Val
                85                  90                  95

GCC AGC ACA GCG TCC ATT TTC AGT GTC TTC ATC CTG TGC ATT GAT CGC      384
Ala Ser Thr Ala Ser Ile Phe Ser Val Phe Ile Leu Cys Ile Asp Arg
            100                 105                 110

TAC CGC TCT GTC CAG CAG CCC CTC AGG TAC CTT AAG TAT CGT ACC AAG      432
Tyr Arg Ser Val Gln Gln Pro Leu Arg Tyr Leu Lys Tyr Arg Thr Lys
        115                 120                 125

ACC CGA GCC TCG GCC ACC ATT CTG GGG GCC TGG TTT CTC TCT TTT CTG      480
Thr Arg Ala Ser Ala Thr Ile Leu Gly Ala Trp Phe Leu Ser Phe Leu
    130                 135                 140

TGG GTT ATT CCC ATT CTA GGC TGG AAT CAC TTC ATG CAG CAG ACC TCG      528
Trp Val Ile Pro Ile Leu Gly Trp Asn His Phe Met Gln Gln Thr Ser
145                 150                 155                 160

GTG CGC CGA GAG GAC AAG TGT GAG ACA GAC TTC TAT GAT GTC ACC TGG      576
Val Arg Arg Glu Asp Lys Cys Glu Thr Asp Phe Tyr Asp Val Thr Trp
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TTC AAG GTC ATG ACT GCC ATC ATC AAC TTC TAC TTG CCC ACC TTG CTC      624
Phe Lys Val Met Thr Ala Ile Ile Asn Phe Tyr Leu Pro Thr Leu Leu
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 992 base pairs

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TTC TAC CTG CCC ACC TTG CTC ATG CTC TGG TTC TAT GCC AAG ATC TAC      192
Phe Tyr Leu Pro Thr Leu Leu Met Leu Trp Phe Tyr Ala Lys Ile Tyr
     50                  55                  60

AAG GCC GTA CGA CAA CAC TGC CAG CAC CGG GAG CTC ATC AAT AGG TCC      240
Lys Ala Val Arg Gln His Cys Gln His Arg Glu Leu Ile Asn Arg Ser
 65                  70                  75                  80

CTC CCT TCC TTC TCA GAA ATT AAG CTG AGG CCA GAG AAC CCC AAG GGG      288
Leu Pro Ser Phe Ser Glu Ile Lys Leu Arg Pro Glu Asn Pro Lys Gly
                 85                  90                  95

GAT GCC AAG AAA CCA GGG AAG GAG TCT CCC TGG GAG GTT CTG AAA AGG      336
Asp Ala Lys Lys Pro Gly Lys Glu Ser Pro Trp Glu Val Leu Lys Arg
            100                 105                 110

AAG CCA AAA GAT GCT GGT GGT GGA TCT GTC TTG AAG TCA CCA TCC CAA      384
Lys Pro Lys Asp Ala Gly Gly Gly Ser Val Leu Lys Ser Pro Ser Gln
        115                 120                 125

ACC CCC AAG GAG ATG AAA TCC CCA GTT GTC TTC AGC CAA GAG GAT GAT      432
Thr Pro Lys Glu Met Lys Ser Pro Val Val Phe Ser Gln Glu Asp Asp
    130                 135                 140

AGA GAA GTA GAC AAA CTC TAC TGC TTT CCA CTT GAT ATT GTG CAC ATG      480
Arg Glu Val Asp Lys Leu Tyr Cys Phe Pro Leu Asp Ile Val His Met
145                 150                 155                 160

CAG GCT GCG GCA GAG GGG AGT AGC AGG GAC TAT GTA GCC GTC AAC CGG      528
Gln Ala Ala Ala Glu Gly Ser Ser Arg Asp Tyr Val Ala Val Asn Arg
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(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(F) TISSUE TYPE: Lung

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Clontech, USA

(B) CLONE: lambda gt11 (16H51b)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..990

(D) OTHER INFORMATION: /partial

/product= "Human Histamine H1 Receptor"

/note= "Coding sequence for the fifth transmembrane region up to the 3' end of the human histamine H1 receptor (lacks the last 22 base pairs)

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  1               5                  10                  15

CAC TTC ATG CAG CAG ACC TCG GTG CGC CGA GAG GAC AAG TGT GAG ACA      96
His Phe Met Gln Gln Thr Ser Val Arg Arg Glu Asp Lys Cys Glu Thr
            20                  25                  30
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(3) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 483 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: Lung

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Clontech, USA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 19..483
(D) OTHER INFORMATION: /partial
/product= "Human Histamine H1 Receptor"

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /note= "Correspond to a part of the primer 3
used for amplification"

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(A) NAME/KEY: primer_bind

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TCT CGA ACG GAC TCA GAT ACC ACC ACA GAG ACA GCA CCA GGC AAA GGC 672
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275 280 285

GCC TTC TGC AAG AAC TGT TGC AAT GAA CAT TTG CAC ATG TTC ACC ATC 912
Ala Phe Cys Lys Asn Cys Cys Asn Glu His Leu His Met Phe Thr Ile
290 295 300

TGG CTG GGC TAC ATC AAC TCC ACA CTG AAC CCC CTC ATC TAC CCC TTG 960
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305 310 315 320

GAT CGC TAC CGC TCT GTC CAG CAG CCC CTC AGG TAC CTT AAG TAT CGT 435
Asp Arg Tyr Arg Ser Val Gln Gln Pro Leu Arg Tyr Leu Lys Tyr Arg
125 130 135

ACC AAG ACC CGA GCC TCG GCC ACC ATT CTG GGG GCC TGG TTT CTC TCT 483
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(4) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1742 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: Lung

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Clontech, USA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1461
(D) OTHER INFORMATION: /product= "Human Histamine H1 Receptor"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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(B) LOCATION: 454..483

(D) OTHER INFORMATION: /note= "Correspond to the primer 4 used for amplification"

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            15                  20                  25

ATG CCC CTG GTG GTG GTC CTG AGC ACT ATC TGC TTG GTC ACA GTA GGG       147
Met Pro Leu Val Val Val Leu Ser Thr Ile Cys Leu Val Thr Val Gly
        30                  35                  40

CTC AAC CTG CTG GTG CTG TAT GCC GTA CGG AGT GAG CGG AAG CTC CAC       195
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    45                  50                  55

ACT GTG GGG AAC CTG TAC ATC GTC AGC CTC TCG GTG GCG GAC TTG ATC       243
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60                  65                  70                  75

GTG GGT GCC GTC GTC ATG CCT ATG AAC ATC CTC TAC CTG CTC ATG TCC       291
Val Gly Ala Val Val Met Pro Met Asn Ile Leu Tyr Leu Leu Met Ser
                80                  85                  90

AAG TGG TCA CTG GGC CGT CCT CTC TGC CTC TTT TGG CTT TCC ATG GAC       339
Lys Trp Ser Leu Gly Arg Pro Leu Cys Leu Phe Trp Leu Ser Met Asp
            95                  100                 105

TAT GTG GCC AGC ACA GCG TCC ATT TTC AGT GTC TTC ATC CTG TGC ATT       387
Tyr Val Ala Ser Thr Ala Ser Ile Phe Ser Val Phe Ile Leu Cys Ile
        110                 115                 120
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          1               5                  10                  15

GGC AAC AAG ACC ACT ATG GCC AGC CCC CAG CTG ATG CCC CTG GTG GTG      96
Gly Asn Lys Thr Thr Met Ala Ser Pro Gln Leu Met Pro Leu Val Val
 5                20                  25                  30

GTC CTG AGC ACT ATC TGC TTG GTC ACA GTA GGG CTC AAC CTG CTG GTG      144
Val Leu Ser Thr Ile Cys Leu Val Thr Val Gly Leu Asn Leu Leu Val
          35                  40                  45
10

CTG TAT GCC GTA CGG AGT GAG CGG AAG CTC CAC ACT GTG GGG AAC CTG      192
Leu Tyr Ala Val Arg Ser Glu Arg Lys Leu His Thr Val Gly Asn Leu
      50                  55                  60

15  TAC ATC GTC AGC CTC TCG GTG GCG GAC TTG ATC GTG GGT GCC GTC GTC      240
    Tyr Ile Val Ser Leu Ser Val Ala Asp Leu Ile Val Gly Ala Val Val
     65                  70                  75                  80

    ATG CCT ATG AAC ATC CTC TAC CTG CTC ATG TCC AAG TGG TCA CTG GGC      288
20  Met Pro Met Asn Ile Leu Tyr Leu Leu Met Ser Lys Trp Ser Leu Gly
                    85                  90                  95

    CGT CCT CTC TGC CTC TTT TGG CTT TCC ATG GAC TAT GTG GCC AGC ACA      336
    Arg Pro Leu Cys Leu Phe Trp Leu Ser Met Asp Tyr Val Ala Ser Thr
25              100                 105                 110

    GCG TCC ATT TTC AGT GTC TTC ATC CTG TGC ATT GAT CGC TAC CGC TCT      384
    Ala Ser Ile Phe Ser Val Phe Ile Leu Cys Ile Asp Arg Tyr Arg Ser
            115                 120                 125
30

    GTC CAG CAG CCC CTC AGG TAC CTT AAG TAT CGT ACC AAG ACC CGA GCC      432
    Val Gln Gln Pro Leu Arg Tyr Leu Lys Tyr Arg Thr Lys Thr Arg Ala
        130                 135                 140

35  TCG GCC ACC ATT CTG GGG GCC TGG TTT CTC TCT TTT CTG TGG GTT ATT      480
    Ser Ala Thr Ile Leu Gly Ala Trp Phe Leu Ser Phe Leu Trp Val Ile
    145                 150                 155                 160
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CCC ATT CTA GGC TGG AAT CAC TTC ATG CAG CAG ACC TCG GTG CGC CGA      528
Pro Ile Leu Gly Trp Asn His Phe Met Gln Gln Thr Ser Val Arg Arg
                165                 170                 175

GAG GAC AAG TGT GAG ACA GAC TTC TAT GAT GTC ACC TGG TTC AAG GTC      576
Glu Asp Lys Cys Glu Thr Asp Phe Tyr Asp Val Thr Trp Phe Lys Val
            180                 185                 190

ATG ACT GCC ATC ATC AAC TTC TAC CTG CCC ACC TTG CTC ATG CTC TGG      624
Met Thr Ala Ile Ile Asn Phe Tyr Leu Pro Thr Leu Leu Met Leu Trp
        195                 200                 205

TTC TAT GCC AAG ATC TAC AAG GCC GTA CGA CAA CAC TGC CAG CAC CGG      672
Phe Tyr Ala Lys Ile Tyr Lys Ala Val Arg Gln His Cys Gln His Arg
    210                 215                 220

GAG CTC ATC AAT AGG TCC CTC CCT TCC TTC TCA GAA ATT AAG CTG AGG      720
Glu Leu Ile Asn Arg Ser Leu Pro Ser Phe Ser Glu Ile Lys Leu Arg
225                 230                 235                 240

CCA GAG AAC CCC AAG GGG GAT GCC AAG AAA CCA GGG AAG GAG TCT CCC      768
Pro Glu Asn Pro Lys Gly Asp Ala Lys Lys Pro Gly Lys Glu Ser Pro
                245                 250                 255

TGG GAG GTT CTG AAA AGG AAG CCA AAA GAT GCT GGT GGT GGA TCT GTC      816
Trp Glu Val Leu Lys Arg Lys Pro Lys Asp Ala Gly Gly Gly Ser Val
            260                 265                 270

TTG AAG TCA CCA TCC CAA ACC CCC AAG GAG ATG AAA TCC CCA GTT GTC      864
Leu Lys Ser Pro Ser Gln Thr Pro Lys Glu Met Lys Ser Pro Val Val
        275                 280                 285

TTC AGC CAA GAG GAT GAT AGA GAA GTA GAC AAA CTC TAC TGC TTT CCA      912
Phe Ser Gln Glu Asp Asp Arg Glu Val Asp Lys Leu Tyr Cys Phe Pro
    290                 295                 300

CTT GAT ATT GTG CAC ATG CAG GCT GCG GCA GAG GGG AGT AGC AGG GAC      960
Leu Asp Ile Val His Met Gln Ala Ala Ala Glu Gly Ser Ser Arg Asp
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305                 310                 315                 320

TAT GTA GCC GTC AAC CGG AGC CAT GGC CAG CTC AAG ACA GAT GAG CAG      1008
Tyr Val Ala Val Asn Arg Ser His Gly Gln Leu Lys Thr Asp Glu Gln
                325                 330                 335

GGC CTG AAC ACA CAT GGG GCC AGC GAG ATA TCA GAG GAT CAG ATG TTA      1056
Gly Leu Asn Thr His Gly Ala Ser Glu Ile Ser Glu Asp Gln Met Leu
            340                 345                 350

GGT GAT AGC CAA TCC TTC TCT CGA ACG GAC TCA GAT ACC ACC ACA GAG      1104
Gly Asp Ser Gln Ser Phe Ser Arg Thr Asp Ser Asp Thr Thr Thr Glu
        355                 360                 365

ACA GCA CCA GGC AAA GGC AAA TTG AGG AGT GGG TCT AAC ACA GGC CTG      1152
Thr Ala Pro Gly Lys Gly Lys Leu Arg Ser Gly Ser Asn Thr Gly Leu
    370                 375                 380

GAT TAC ATC AAG TTT ACT TGG AAG AGG CTC CGC TCG CAT TCA AGA CAG      1200
Asp Tyr Ile Lys Phe Thr Trp Lys Arg Leu Arg Ser His Ser Arg Gln
385                 390                 395                 400

TAT GTA TCT GGG TTG CAC ATG AAC CGC GAA AGG AAG GCC GCC AAA CAG      1248
Tyr Val Ser Gly Leu His Met Asn Arg Glu Arg Lys Ala Ala Lys Gln
                405                 410                 415

TTG GGT TTT ATC ATG GCA GCC TTC ATC CTC TGC TGG ATC CCT TAT TTC      1296
Leu Gly Phe Ile Met Ala Ala Phe Ile Leu Cys Trp Ile Pro Tyr Phe
            420                 425                 430

ATC TTC TTC ATG GTC ATT GCC TTC TGC AAG AAC TGT TGC AAT GAA CAT      1344
Ile Phe Phe Met Val Ile Ala Phe Cys Lys Asn Cys Cys Asn Glu His
        435                 440                 445

TTG CAC ATG TTC ACC ATC TGG CTG GGC TAC ATC AAC TCC ACA CTG AAC      1392
Leu His Met Phe Thr Ile Trp Leu Gly Tyr Ile Asn Ser Thr Leu Asn
    450                 455                 460
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CCC CTC ATC TAC CCC TTG TGC AAT GAG AAC TTC AAG AAG ACA TTC AAG 1440
Pro Leu Ile Tyr Pro Leu Cys Asn Glu Asn Phe Lys Lys Thr Phe Lys
465 470 475 480

AGA ATT CTG CAT ATT CGC TCC TAAGGGAGGC TCTGAGGGGA TGCAACAAAA 1491
Arg Ile Leu His Ile Arg Ser
485

TGATCCTTAT GATGTCCAAC AAGGAAATAG AGGACGAAGG CCTGTGTGTT GCCAGGCAGG 1551

CACCTGGGCT TTCTGGAATC CAAACCACAG TCTTAGGGGC TTGGTAGTTT GGAAAGTTCT 1611

TAGGCACCAT AGAAGAACAG CAGATGGCGG TGATCAGCAG AGAGATTGAA CTTTGAGGAG 1671

GAAGCAGAAT CTTTGCAAGA AAGTCAGACC TGTTTCTTGT AACTGGGTTC AAAAAGAAAA 1731

AAAAAAAAAA A 1742

(5) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..52
(D) OTHER INFORMATION: /note= "(primer 1). 5' end coding sequence corresponding to bovine cDNA used for amplification."

(ix) FEATURE:

(A) NAME/KEY: primer_bind

(B) LOCATION: 5..52

(D) OTHER INFORMATION: /note= "Corresponds to bases 1 to 48 in SEQ ID NO: 1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TACAAAGCTT ACCATGACCT GTCCCAACTC CTCCTGCGTC TTCGAAGACA AG 52

(6) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic cDNA

(ix) FEATURE:

(A) NAME/KEY: primer_bind

(B) LOCATION: 1..51

(D) OTHER INFORMATION: /note= "(primer 2); part of the complementary DNA sequence of the 5th transmembrane region of the bovine cDNA used for amplification".

(ix) FEATURE:

(A) NAME/KEY: primer_bind

(B) LOCATION: 8..51

(D) OTHER INFORMATION: /note= "corresponds to the complementary bases 607 to 649 in SEQ ID No: 1".

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AGCCTTGTAG ATCTTGGCAT AGAACCAGAG CATGAGCAAG GTGGGCAAGT A 51

(7) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /note= "(primer 3); 5' leader non coding sequence of the bovine cDNA used for amplification/ corresponds to bases 1 to 21 in SEQ ID NO: 3."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GAGGCTACAC TTGTGCCAAT G 21

(8) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic cDNA

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..30
(D) OTHER INFORMATION: /note= "(primer 4); complementary sequence from human cDNA coding for 4th transmembrane region, used for amplification/ corresponds to bases 450 to 483 in SEQ ID NO: 3."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AGAGAGAAAC CAGGCCCCCA GAATGGTGGC 30

(9) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..43
(D) OTHER INFORMATION: /note= "(primer 5); 5' sequence of human cDNA"

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 14..43

(D) OTHER INFORMATION: /note= "corresponds to bases 1 to 30 in SEQ ID NO: 4."

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 11..13
(D) OTHER INFORMATION: /note= "Consensus sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TACAAAGCTT CCAATGAGCC TCCCCAATTC CTCCTGCCTC TTA 43

(10) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic cDNA

(ix) FEATURE:
(A) NAME/KEY: primer_bind
(B) LOCATION: 1..33
(D) OTHER INFORMATION: /note= "(primer 6); used for amplification/ complementary sequence coding for 1st intracellular region of human cDNA/ corresponds to bases 157 to 189 in SEQ ID NO: 4."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTTCCCCACA GTGTGGAGCT TCCGCTCACT CCG 33

CLAIMS

1. An isolated nucleic acid molecule encoding a human histamine H_1 receptor.

2. An isolated DNA molecule encoding a human histamine H_1 receptor.

3. A DNA molecule as claimed in claim 2, comprising a coding sequence substantially the same as the coding sequence shown in SEQ ID No:4.

4. A DNA molecule as claimed in claim 2, which is a cDNA molecule.

5. An isolated protein which is a human histamine H_1 receptor.

6. An isolated protein, as claimed in claim 5, comprising substantially the same amino acid sequence as the amino acid sequence shown in SEQ ID No:4.

7. A vector comprising a DNA molecule as claimed in any of claims 2 to 4.

8. A vector adapted for expression in a mammalian cell

which comprises a DNA molecule as claimed in any of claims 2 to 4 and the regulatory elements necessary for expression of the DNA in the mammalian cell.

9. A vector adapted for expression in a bacterial cell which comprises a DNA molecule as claimed in any of claims 2 to 4 and the regulatory elements necessary for expression of the DNA in the bacterial cell.

10. A vector adapted for expression in a yeast cell which comprises a DNA molecule as claimed in any of claims 2 to 4 and the regulatory elements necessary for expression of the DNA in the yeast cell.

11. A mammalian cell comprising an expression vector as claimed in claim 8.

12. A transfected CHO cell comprising an expression vector as claimed in claim 8.

13. An antibody directed to a human histamine H_1 receptor.

14. An antibody directed to an epitope of a human histamine H_1 receptor present on the surface of a cell and having an amino acid sequence substantially the same as the amino acid sequence shown in SEQ ID No:4, or a sequential subset

thereof.

15. A method for determining whether a ligand can bind to a human histamine H_1 receptor, which comprises contacting a cell as claimed in claim 11 or 12 with the ligand, under conditions permitting binding of a ligand known to bind a histamine H_1 receptor, detecting the presence of any of the ligand bound to a human histamine H_1 receptor, and thereby determining whether the ligand binds to a human histamine H_1 receptor.

16. A method of detecting the presence of mRNA coding for a human histamine H_1 receptor in a cell, which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a DNA as claimed in claim 3 under hybridizing conditions, detecting the presence of mRNA hybridized to the DNA, and thereby detecting the presence of mRNA encoding a human histamine H_1 receptor in the cell.

17. A method of screening drugs to identify a drug or drugs which specifically interact with, and bind to, a human histamine H_1 receptor on the surface of a cell, which comprises contacting a cell as claimed in claim 11 or 12 with at least one drug, determining whether the drug or drugs bind to the cell, and thereby identifying a drug or drugs which specifically interact with, and bind to, a human

H_1 receptor.

18. A DNA probe useful for detecting a nucleic acid encoding a human histamine H_1 receptor, which comprises a nucleic acid molecule of at least about 15 nucleotides and having a sequence complementary to a coding sequence included within the DAN sequence shown in SEQ ID No: 4.

19. A method of detecting the presence of a human H_1 receptor on the sruface of a cell, which comprises contacting the cell with a monoclonal or serum-based antibody as claimed in claim 14 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell and thereby the presence of a human H_1 receptor on the surface of the cell.

20. An isolated nucleic acid molecule, isolated protein or antibody substantially as hereinbefore described in the Examples or sequence listing.

21. A vector comprising a nucleic acid molecule as claimed in claim 20 and substantially as hereinbefore described in the Examples, or as shown in the drawings.

22. A method of determining whether a ligand can bind to a human histamine H_1 receptor, detecting the presence of mRNA

coding for a human histamine H_1 receptor, screening drugs for capability of interaction with a human histamine H_1 receptor, or detecting the presence of a human H_1 receptor on the surface of a cell, substantially as hereinbefore described in the Examples.

23. A DNA probe for detecting nucleic acid encoding human histamine H_1 receptor, substantially as hereinbefore described in the Examples or as shown in the sequence listing.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search report) - 45 -

Application number
GB 9322353.5

Relevant Technical Fields

(i) UK Cl (Ed.5) C3H (HB7P, HB7M, HA5)

(ii) Int Cl (Ed.M) C07K 15/06, C12N 15/12

Search Examiner
Dr N CURTIS

Date of completion of Search
23 DECEMBER 1994

Databases (see below)
(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE: WPI, BIOTECH (DIALOG)

Documents considered relevant following a search in respect of Claims :-
1 TO 23

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages	Relevant to claim(s)
P, X	Eur. J. Biochem, Volume 224, 1994, pages 489 to 495 (MOGUILEVSKY ET AL)	1 to 23
P, X	Biochemical and Biophysical Research Communications, Volume 201, No. 2, 1994, pages 894 to 901	1 to 19
P, X	J. Allergy Clin. Immunol., Volume 93, No. 1, part 2, 1993, page 215, Abstract 314 (CHOWDHURY ET AL)	1 to 19
P, X	Biochemical and Biophysical Research Communications, Volume 197, No. 3, 1993, pages 1601 to 1608 (DE BACKER ET AL)	1 to 19
Y	Biochemical and Biophysical Research Communications, Volume 190, No. 1, 1993, pages 294 to 301 (FUJIMOTO ET AL)	1 to 19
Y	Biochemical and Biophysical Research Communications, Volume 178, No. 3, 1991, pages 1386 to 1392 (GRANTZ ET AL)	1 to 19
Y	Proc. Natl. Acad. Sci, USA, Volume 88, 1991, pages 11515 to 11519 (YAMASHITA ET AL)	1 to 19

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).